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LIBERATION OF HISTAMINE AND FORMATION OF LYSOCITHIN-LIKE SUBSTANCES BY COBRA VENOM

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WE have recently shown [1937*a*] that cobra venom causes the release of histamine from perfused lungs of cats and guinea-pigs. The experiments reported in the present paper concern the liberation of histamine from liver and lungs of other animals and the manner in which this effect may be produced.

One of the actions of cobra venom is to cause haemolysis; this is not a direct effect of the venom on the red blood corpuscle but results from its action on lecithin. Kyes [1904] in Ehrlich's laboratory found that if cobra venom is brought into contact with lecithin a haemolytic substance is produced. Delezenne & Fourneau [1914] showed that this substance is a derivative of lecithin. The cobra venom contains a lecithinase which splits off oleic acid from lecithin. The resulting rest, lysocithin, is powerfully haemolytic. It appeared conceivable that the formation of lysocithin might account for effects of the venom, other than haemolysis and that its formation in the tissues might make the cells permeable to their histamine. Although this theory has not hitherto been put forward in this form, observations of Belfanti [1925, 1928], Guerrini [1925] and Houssay and his co-workers [Houssay, 1930] suggest that the formation of lysocithin may play a dominant role in the symptomatology of snake venom poisoning. They showed that the injection of lysocithin into animals caused symptoms resembling in some respects those caused by snake venoms. On the other hand, Belfanti's results on the action of snake venoms on emulsions made from different organs seemed to exclude the formation of lysocithin in all the tissues tested (heart, liver, kidney,

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spleen) except brain. If the conclusions drawn from these experiments were correct the effects of snake venom on isolated perfused organs would be independent of the formation of lysocithin.

In our previous experiments on perfused lungs we have found that the venous perfusate after the injection of venom caused not only a rapid histamine-like contraction but sometimes also a delayed and slow contraction of the isolated jejunum of the guinea-pig. We have encountered a similar phenomenon in experiments on the effect of bee venom on the perfused guinea-pig's lung and dog's liver [1937*b*]. It was tempting to assume that lysocithin from lecithin or substances formed in the tissues from lipins other than lecithin might account for this slow contraction. A comparison of the unknown active principle with lysocithin was therefore indicated.

Our experiments are divided into two parts: the action of cobra venom on perfused lungs and livers and a comparison of some of the pharmacological effects of the active substance or substances formed by venom in perfused organs with those formed *in vitro* by venom on lecithin.

Methods

We perfused the lungs and livers of dogs and monkeys (*Macacus rhesus*) with Tyrode solution using the method already described [1937*a*, *b*], the rate of perfusion being 6–12 c.c. per min. Whereas only a small portion of the dog's liver was used almost the whole of the monkey's liver, weighing 60–80 g., was perfused. The venous perfusate was assayed for histamine on a piece of guinea-pig's jejunum suspended in a 3 c.c. bath. Protein in the perfusate was roughly estimated by Esbach's method.

Extracts of organs. Saline extracts were made by grinding the organs with powdered quartz in Tyrode solution [for details see 1937*a*]. The extracts were boiled before testing with the exception of some of the monkey's liver which were also tested unboiled. Extracts from the monkey's liver were also made in absolute methyl alcohol. The organ ground with powdered quartz was repeatedly extracted with alcohol, about 10 c.c. being used per gram of tissue. The extract was filtered, taken to dryness on the water bath and re-extracted with absolute methyl alcohol. This was again filtered. The final filtrate was made up to such a volume that 1 c.c. corresponded to 1 g. of liver. For use, measured amounts of the extract were evaporated and the residue taken up in a given volume of physiological saline solution. Methyl alcoholic extracts made from monkey's liver after perfusion and the injection of large doses of cobra venom will be termed "envenomed liver".

Preparation of "lysocithin". Several batches of lysocithin were prepared by the action of cobra venom on egg lecithin which was made for us by our colleague, H. F. Holden. One gram of lecithin was emulsified and suspended in 1300 c.c. of buffered phosphate solution at *p*H 7·7. The solution contained 400 c.c. *N*/5 KH_2PO_4 and 70 c.c. 1·015 *N* NaOH per l. The suspension of lecithin was brought to 37° C. in a water bath and 0·8–1·2 g. of cobra venom dissolved in 20 c.c. of physiological saline solution was added. Samples of the mixture were at once tested in various dilutions for their capacity to haemolyse washed sheep red corpuscles, which are not haemolysed by cobra venom in any concentration but undergo immediate haemolysis with lysocithin. Further tests were made from time to time, and when no further increase in the titre of lysocithin was to be seen the mixture was taken to dryness on a boiling water bath and extracted by repeated rubbing up with pure absolute methyl alcohol. The methyl alcohol extract was filtered, taken to dryness and again taken up in absolute methyl alcohol. This extract was filtered and made up to 50 or 100 c.c. so that 1 c.c. of our extract contained lysocithin equivalent to 10 or 20 mg. of lecithin. Although this process of concentration involved evaporation on a boiling water bath for several hours, twice extracting with methyl alcohol and twice filtering, there was no appreciable loss of haemolytic activity. For use, the alcoholic solution was evaporated to dryness on a water bath and emulsified in physiological saline. The appearance of these emulsions varied somewhat. Two batches yielded a clear gelatinous watery solution, but more usually a fine opaque suspension which did not sediment and readily passed through a No. 1 Whatman filter paper without loss of activity was obtained.

We made no attempt to prepare pure lysocithin. Our extracts may, therefore, have contained other active substances formed by the action of cobra venom on lecithin or, since our sample of lecithin was probably not pure, from impurities present in it. For this reason, where we refer to our extracts of lysocithin we use inverted commas.

We shall express the amount of "lysocithin" as equivalents of pure lecithin. There was some variation in the activity of different batches. Comparison of the activity of "lysocithin" in different experiments and pharmacological tests were, therefore, always made with a single batch.

Pharmacological tests. Uterus of the virgin guinea-pig; jejunum of the young rat. The muscles were suspended in a 3 c.c. bath in oxygenated anaphylactic Ringer's solution (NaCl 0·9%, KCl 0·042%, $CaCl_2$ 0·012%, glucose 0·1%, $NaHCO_3$ 0·05%). *Cat's heart:* Gunn's modification [1913] of Langendorff's perfusion method was used. The

water in the heating jacket was supplied from a large thermostat which was also used to warm the Ringer's solution (NaCl 0·9%, KCl 0·042%, $CaCl_2$ 0·024%, glucose 0·1%, $NaHCO_3$ 0·01%). The thermostat was supplied with a continuous flow of tap water from a constant level and the outflow to the water jacket was also supplied from a constant level in the thermostat. With this arrangement the temperature of the perfusing fluid, despite large variations in coronary flow, did not alter more than 0·5° C. during the course of an experiment. The reservoir of the cold Ringer's solution was 80–90 cm. above the heart and was oxygenated by passing "carbogen" (5% CO_2 in O_2) through a coarse porous filter immersed in it. The Ringer's solution was brought to a *p*H of about 7·5 (estimated by colorimetric comparison using phenol red as an indicator) by bubbling CO_2 through it. All injections were made in a volume of 1 c.c. directly into the aortic cannula. *Sheep's red corpuscles:* The cells were washed three times in 0·9% NaCl solution and used in a 5% suspension. The haemolytic system was 0·2 c.c. (0·1 c.c. of cells and 0·1 c.c. of the solutions tested which were put up in doubling dilutions). The tests were made at 37° C. in a water bath and the haemolysis times determined by continuous or frequent observations.

Results

Part 1. Effects of cobra venom on perfused lungs and liver

In addition to the changes caused by the venom in these organs there appeared in the venous perfusate, protein, histamine, a substance (or substances) which causes a delayed and slow contraction of the guinea-pig's jejunum and, in the case of the liver, pigments.

A. *Identification of histamine*

The identification of histamine in perfusates and organ extracts was the same in all experiments. Since the liver contains acetylcholine and choline it was possible that part of the histamine-like activity on the gut of the liver extracts and perfusates might be caused by these substances. Acetylcholine was excluded by treating the solutions with alkali, which did not diminish their activity. This was to be expected since the conditions under which extracts and perfusates were obtained favoured hydrolysis of acetylcholine. The presence of choline in effective concentrations was improbable because the amounts of extract necessary for the assay usually corresponded to between 0·5 and 1 mg. of fresh tissue, and because perfusates could be tested in high dilution. Choline was excluded by the fact that the activity persisted after atropine.

The identification as histamine of the substance *in extracts* which caused rapid contraction of the gut rested upon pharmacological assays in which its activity was compared quantitatively with histamine in three different tests and upon the fact that histamine has been isolated from livers and lungs, identified chemically and made responsible for the histamine-like activity of extracts [Hanke & Koessler, 1924; Lucas, 1926; Best, Dale, Dudley & Thorpe, 1927]. The identification of this active substance *in perfusates* rested upon pharmacological assay by the same tests, and upon the demonstration that it was part of the tissue histamine, which diminished to the same extent as the activity in the perfusate increased. We shall therefore refer to it as histamine. In Fig. 1 is illustrated the assay of a sample of perfusate collected from a piece of perfused dog's liver in the second hour after the intraportal injection of 18 mg. of cobra venom. The stimulating action of the boiled perfusate on the guinea-pig's jejunum corresponded to that of histamine 1 : 500,000. In the upper tracings of Fig. 1 the response of the plain muscle to 0·2 c.c. of a 1 : 10 dilution of this sample is "bracketed" by responses to 0·2 c.c. of two histamine solutions, 1 : 6 million and 1 : 4 million. In the middle and lower tracings the effect of 0·5 c.c. of the undiluted sample is "bracketed" by responses to 0·5 c.c. of two histamine solutions 1 : 600,000 and 1 : 400,000. The middle tracings show the depressor action of these solutions on the cats' blood pressure by intravenous injection. The lower tracings show the pressor responses resulting from output of adrenaline in the same cat, after evisceration and ligature of the aorta and vena cava inferior distal to the suprarenals, the solutions

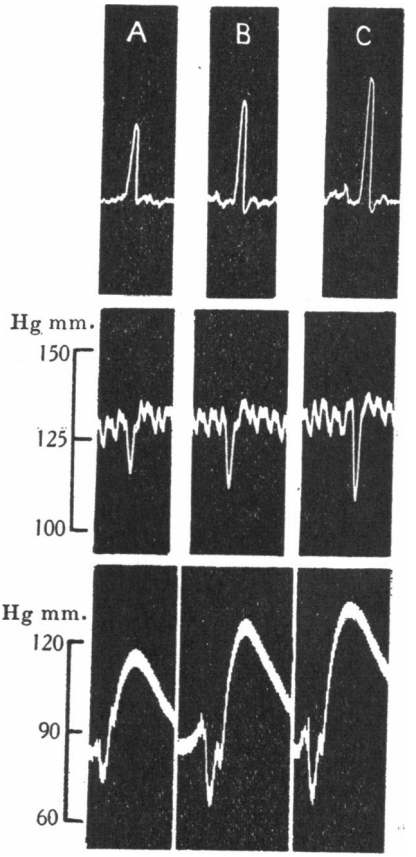

Fig. 1. Records from guinea-pig's jejunum (upper tracing) and cat's arterial blood pressure (middle and lower tracings). A and C, histamine; B, perfusate from envenomed dog's liver. (Details in text.)

being injected into a cannula tied into the central stump of the coeliac axis. It will be seen that in all these tests the undiluted perfusate was stronger than histamine, 1:600,000, and weaker than histamine, 1:400,000.

B. *Dog's lungs*

We have shown [1937*b*] that the histamine equivalent of the lungs in different dogs varies between 20 and 220 μg. per g.; that isolated lobes may be perfused with Tyrode solution for many hours without gross changes in their appearance or great accumulation of fluid in them; that the venous perfusate remains free from detectable amounts of histamine and that no loss of histamine occurs from the lungs during prolonged perfusion.

Two or three minutes after the injection of venom (10 mg. or more), the respiratory movements of the artificially ventilated lobes diminish or cease; they can, however, be restored by increasing the ventilation. The venous outflow slows down and may stop without diminution of the inflow; tremendous swelling occurs and the lungs become heavy and glassy in appearance. In the course of some 10 min., fluid, in an amount corresponding to two or three times the weight of the perfused lobes, may accumulate and some may appear in the trachea during expiration. In contrast to experiments on perfused guinea-pig's lungs there is little leakage from the surface, but the accumulated fluid is easily squeezed out from the cut veins at the hilus by strong over-ventilation for a minute or two. If normal ventilation is restored the lungs again become swollen and fluid can be squeezed out by over-ventilation. The fluid is turbid, contains coagulable protein, histamine and a substance (or substances) which causes a slow contraction of the isolated jejunum of the guinea-pig and temporary changes in the reactivity of the muscle to histamine.

Figs. 2 and 3 illustrate the output of histamine and of coagulable protein in a typical experiment. The upper and lower lobes of the left lung from a 7·7 kg. dog were perfused. These weighed about 25 g. The right lung contained 40μg. of histamine per g. which indicated a content of about 1 mg. histamine for the perfused lobes. Perfusion at a rate of 8–10 c.c. per min. was continued for 40 min. before the injection of 12 mg. of cobra venom in 1·5 c.c. was made. The perfusate was tested in Fig. 2 on the guinea-pig's jejunum. The perfusate (0·4 c.c.) collected before the injection of venom was without effect (at B). At C to H, 0·2 c.c. of the successive samples after the injection were tested. That tested at C was collected before squeezing out the fluid from the lung by over-ventilation.

Fig. 3 gives the output in μg. per min.; it reached its maximum in the second half hour after the injection, 454 μg. being assayed in this sample. The total output during 2½ hr. was 942 μg. and another 4 μg. were found in the fluid draining from the lung after the perfusion was stopped ("drainage

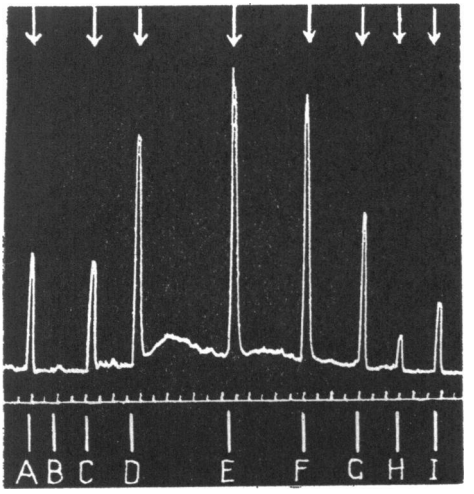

Fig. 2.

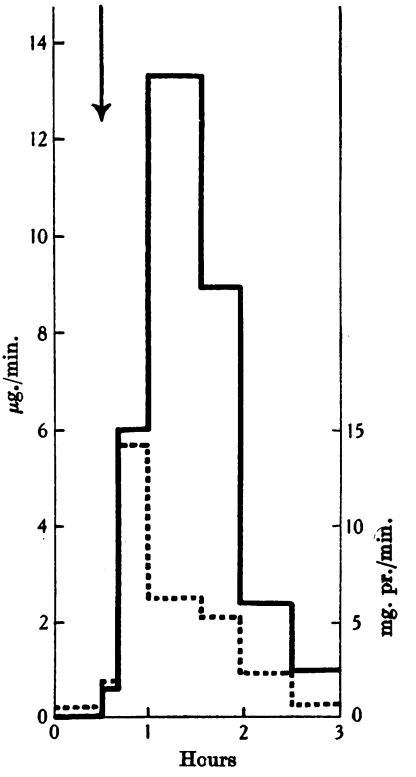

Fig. 3.

Fig. 2. Record from guinea-pig's jejunum desensitized to cobra venom. At A and I, 0·04 μg. histamine; at B to H, boiled perfusate from dog's lung. At the arrows, bath washed out. (Details in text.)

Fig. 3. Output of histamine (continuous line) and of protein (dotted line) from perfused dog's lung. At the arrow 12 mg. cobra venom. Ordinates on the left, histamine in μg. per min. and on the right, protein in mg. per min. Abscissae, time in hours.

fluid"). The tissue should now have been practically depleted of its histamine and this was found to be the case. The lung, after drainage, weighing 44 g. contained 22 μg. of histamine.

The concentration of coagulable protein was strongest in the samples collected 10–30 min. after the injection of the venom, the maximum out-

put preceding that of histamine (Fig. 3). In experiments on the effect of bee venom on the perfused lung of the dog we found [1937*b*] a parallelism between the histamine and protein concentration of the different samples of perfusate. In the present experiment the rate of perfusion was greater, which may account for the difference in the results.

In addition to the immediate rapid contraction of the guinea-pig's jejunum, perfusate often caused a delayed slow contraction. Figs. 4 and 5 show different types of this contraction complicating the rapid effect of histamine. At A (Fig. 4) the secondary contraction set in before the gut

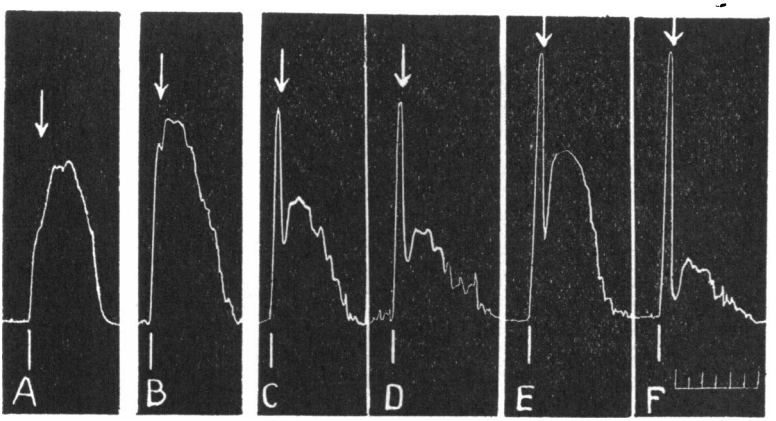

Fig. 4. Records from guinea-pig's jejunum desensitized to cobra venom (three preparations). 0·2 c.c. perfusate from envenomed dog's lungs (A to D) and liver (E and F). C to F, on the same preparation (D and F boiled). Time in 30 sec. (Details in text.)

had started to relax from its histamine contraction; at B the distinction between the histamine and secondary contractions is better defined. At C and D after washing out the perfusate the secondary contraction set in before the muscle had relaxed completely, and in Fig. 5 it appeared merely to delay the normal relaxation of the muscle. Further variations in the response of the muscle are shown in Fig. 8 for perfusate collected from a monkey's lung. The different types of contraction are determined by the sensitivity of the preparation to histamine and to the substance causing the slow contraction, by the concentrations of both substances in the samples tested and by the length of time allowed in contact with the muscle. When a sample of perfusate is brought to the boiling point and then cooled, its action in causing the secondary contraction is somewhat reduced. In Fig. 4 are shown the effects of a sample of perfusate before (at C) and after boiling (at D). When perfusate is repeatedly tested (ten

to twenty times) on the same preparation and particularly when large amounts of perfusate are used, the muscle may gradually become less sensitive to the slowly contracting principle although its response to histamine is not weakened. In this condition the preparation is best suited for the assay of histamine in the perfusate. In Fig. 2, for instance, the assay was carried out on a preparation which had been used for several hours and had become somewhat insensitive to the slowly contracting substance. Furthermore, the samples of perfusate were boiled before testing and left in contact with the muscle for 5–7 sec. only.

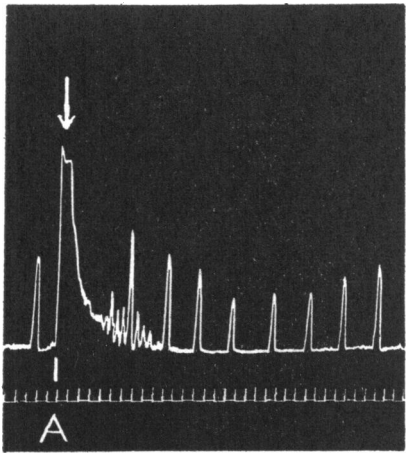

Fig. 5. Record from guinea-pig's jejunum desensitized to cobra venom. At A, 0·2 c.c. perfusate from envenomed dog's lung. The remaining responses are to 0·04 μg. histamine. Time in 30 sec.

When the muscle has relaxed from the secondary contraction it is sometimes, at first, more, and later, less sensitive to histamine. The former state lasts only a few minutes, the latter for times up to 20 min. This is illustrated in Fig. 5. Frequently only one of these states is observed.

The principle causing the slow contraction and the changes in excitability of the muscle is formed by the action of the venom. This becomes apparent when the effects on the guinea-pig's jejunum of extracts of normal and envenomed lungs are compared. Normal lung usually causes only a histamine effect though there may be a slight after-contraction; with envenomed lung the histamine effect is followed by a pronounced after-contraction. In Fig. 6, A gives the effect of boiled saline extract of 5 mg. of normal lung, and B that of 10 mg. from the same

lung after injection of 20 mg. of cobra venom and 90 min. perfusion. Since the lung weight had doubled the doses correspond. It will be seen, at A, that the muscle, which had begun to relax while the extract was present in the bath, rapidly reached its original length when the Tyrode solution was changed after 30 sec. At B, the initial histamine contraction was weaker than that at A, part of the lung histamine having been liberated, but there was an after-contraction from which the muscle

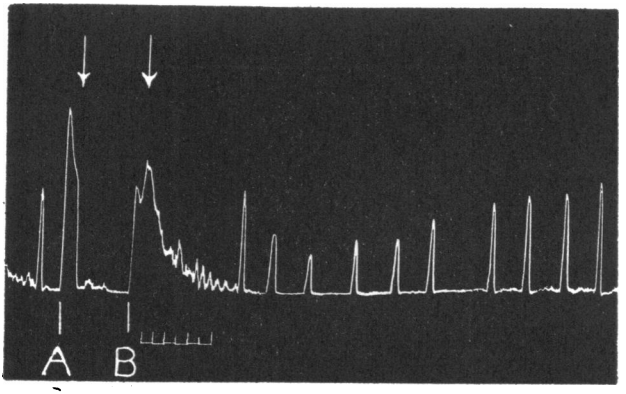

Fig. 6. Record from guinea-pig's jejunum desensitized to 100 μg. cobra venom. At A, extract of normal, at B, of envenomed dog's lung. The remaining responses are to 0·04 μg. histamine. At the arrows, bath washed out. Time in 30 sec. (Details in text.)

recovered gradually when the Tyrode solution was changed. The after-contraction caused by envenomed lung was followed by changes in excitability of the muscle similar to those observed after venous perfusate. In Fig. 6 there was a period of decreased reactivity to histamine.

C. *Monkey's lungs*

We have perfused the left lungs of two monkeys; the results were essentially the same as those described for the dog's lung. The lungs weighed about 15 g. and contained 80 and 100 μg. of histamine per g. respectively. Before the injection of venom the perfusate contained no detectable amount of histamine. After the injection the respiratory movements diminished, the outflow became slower without diminution of the inflow and the lung became swollen and glassy in appearance. The accumulated fluid could be squeezed out by over-ventilation. Histamine, protein and the substance (or substances) which caused slow contraction and changes of excitability of the isolated guinea-pig's jejunum appeared in the perfusate.

Fig. 7 illustrates the histamine and protein output in one of these experiments after the injection of 10 mg. of cobra venom. The histamine output rose to 11 μg. per min. in the first $\frac{1}{2}$ hr. and then fell again rapidly. The total output was 872 μg. and 25 μg. were assayed in the "drainage fluid". This corresponded to 87 % of the histamine available in the perfused lung. In the other experiment 2 mg. of cobra venom in 1 c.c. of saline

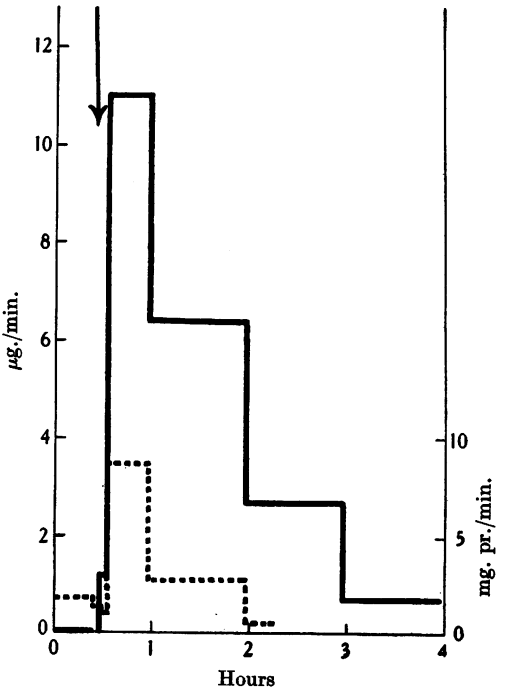

Fig. 7. Output of histamine (continuous line) and of protein (dotted line) from perfused monkey's lung. At the arrow, 10 mg. cobra venom (in 1 c.c.). Ordinates and abscissae as in Fig. 3.

solution were injected. The histamine output was 698 μg. during 2$\frac{1}{2}$ hr. perfusion and 40 μg. were found in the "drainage fluid". The total output of 738 μg. amounted to 53 % of the lung histamine, the lung after drainage yielding 664 μg. by extraction.

The substance causing after-contraction appeared to be present in greater amount than in the perfusate from envenomed dog's lungs; it was most concentrated in perfusate collected during the first 1 or 2 hr. after the injection of venom. In Fig. 8 are shown the effects on the gut of perfusate from the experiments of Fig. 7. At A, 0·04 c.c. of the sample

collected 35–95 min., and at B, 0·2 c.c. of that collected during the last hour of perfusion, were added to the bath. The immediate sharp contractions were due to histamine present in concentrations of about 1 : 1 million

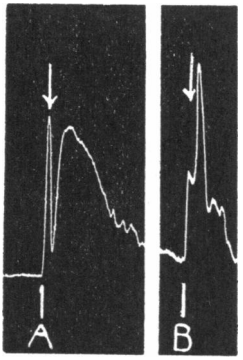

Fig. 8. Record from guinea-pig's jejunum desensitized to cobra venom. At A and B, perfusate from envenomed monkey's lung. At the arrows, bath washed out. (Details in text.)

and 1 : 10 millions respectively. The after-contractions were followed by evanescent changes in excitability similar to those observed in experiments with perfused dog's lungs.

D. *Dog's liver*

We have shown [1937*b*] that the dog's liver contains between 20 and 110μg. of histamine per g.; that it may be perfused for many hours without detectable loss of its histamine and that the venous perfusate is usually devoid of activity. In a few experiments we found that perfusate collected at the beginning of the perfusion had a slight histamine-like action on the gut. When perfusion was continued for 1 or 2 hr. this activity diminished and eventually disappeared.

Intraportal injection of a single large dose of venom (5 *mg. or more*) *into the perfused liver.* After the injection the liver becomes somewhat swollen and loses its brownish colour. Depigmentation seems to affect first the region of the lobule near Glisson's capsule giving the liver a reticulated appearance, but gradually the whole lobule is affected. If the venom is evenly injected all parts of the organ participate in this change, otherwise those which are not reached by the venom retain their brown colour. The venous perfusate assumes a brownish yellow tint. This is deepest in the samples collected during the first few minutes after the injection; 20–50 min. later the brownish yellow colour has usually disappeared from

the perfusate. When the blood has not been completely washed out from the liver, the early samples collected after the injection contain blood pigments. The perfusate, after the injection becomes turbid, contains protein, histamine and the substance (or substances) which causes slow contraction and the changes in reactivity of the gut.

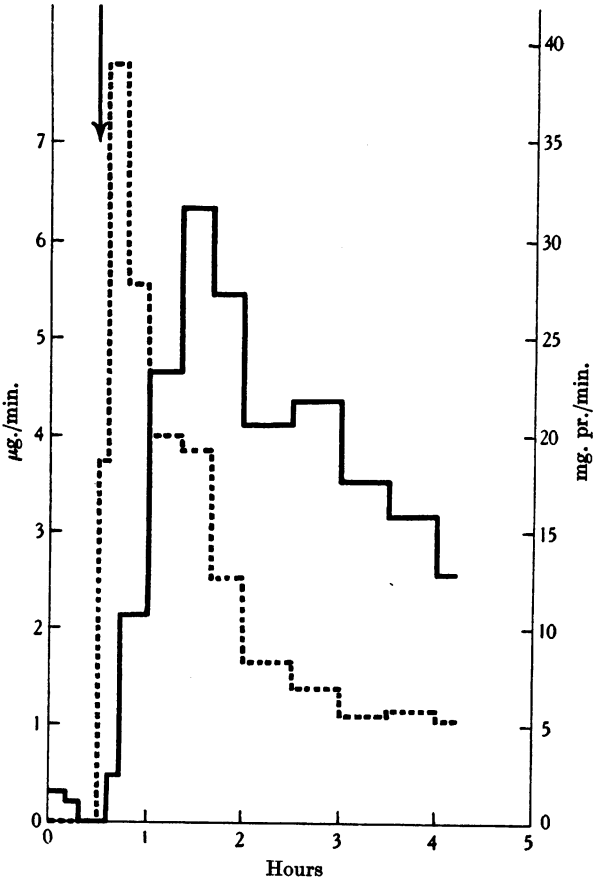

Fig. 9. Output of histamine (continuous line) and of protein (dotted line) from perfused dog's liver. At the arrow, 7·5 mg. cobra venom. Ordinates and abscissae as in Fig. 3.

In Fig. 9 is shown the output of protein, and of histamine from a piece of liver weighing 60 g. The output of protein reaches its maximum in the second sample of perfusate collected 5–15 min. after the injection amounting to 390 mg.; the total output during 220 min. perfusion was 2·8 g. The maximum output of protein precedes that of histamine, but

follows that of liver pigment. The loss of protein from the liver was apparent when saline extracts of perfused normal and envenomed liver were boiled. The protein in the former was coagulated in large flakes and in the latter in fine flakes.

The liver from the experiment of Fig. 9 contained 36 μg. of histamine per g. The perfusate, before the injection, had some histamine-like activity, which disappeared as perfusion proceeded. Five minutes after the injection of venom histamine reappeared, increased during the first hour and then decreased again. The total output during 3¼ hr. perfusion amounted to 822 μg., and 24 μg. were assayed in the "drainage fluid". This represented about 40 p.c. of the liver histamine.

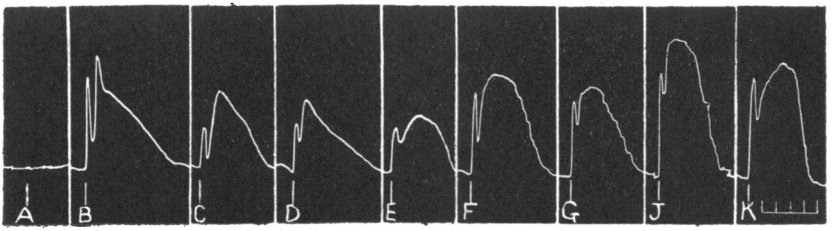

Fig. 10. Record from guinea-pig's jejunum desensitized to cobra venom. Perfusate from dog's liver before (A) and after cobra venom (B to K). Time in 30 sec. (Details in text.)

The slow after-contraction of the guinea-pig's jejunum and the ensuing changes in reactivity of the muscle were pronounced. The concentration in the perfusate of the substance (or substances) responsible for these effects was dependent upon the dose of venom injected and upon the rate of perfusion, being stronger with large doses and slow perfusion. Fig. 10 shows the effects on the gut of different amounts of successive samples of perfusate from a piece of liver weighing 70 g. before (at A) and after (at B to K) the intraportal injection of 18 mg. of venom. The rate of perfusion was 1 c.c. per min. At A and B, 0·2 c.c., at C and D, 0·04 c.c., at E, F and G, 0·01 c.c., and at J and K, 0·02 c.c. were tested. B was from a sample 10–20 min., C, 20–30 min., D, 20–60 min., E in the second, F in the third, G in the fourth, J in the fifth and K in the sixth hour after the injection. The concentration of the slowly contracting substance first increased and then decreased again following roughly the same course as the output of histamine. The perfusate after boiling lost much of its action in causing slow contraction, whereas the histamine-like activity remained unimpaired. Boiling had more effect upon perfusates from envenomed liver than on those from envenomed lungs. This is illustrated in

Fig. 4, which shows the effects of unboiled (C and E) and boiled (D and F) perfusates from envenomed lungs and liver of a dog. In spite of this difference in behaviour the after-contraction is probably caused by the same substance in both perfusates. Perfusate from the liver contains more protein than that from the lung and differences in the reduction of activity by boiling may result from adsorption to protein coagulated by heat (*vide infra*). The protein concentration of the sample tested at Fig. 4E was about five times as great as that of the sample tested at C.

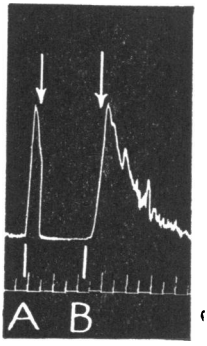

Fig. 11. Record from guinea-pig's jejunum desensitized to cobra venom. Boiled saline extract of normal (A) and envenomed dog's liver (B). Time in 30 sec. (Details in text.)

A comparison of the effects of extracts of normal and envenomed livers shows that the substance causing the slow contraction must be formed in the organ by the action of the venom. Saline extracts of a few milligrammes of normal liver usually cause only an immediate histamine effect, although there may in some instances be a slight after-contraction. With extracts from envenomed liver the histamine effect is weaker, but there is a strong after-contraction, which, if the histamine loss has been great, may be the sole response. Such a result is shown in Fig. 11. At A was tested 2 mg. of normal and at B 2 mg. from the same liver extracted 90 min. after the injection of venom. The contraction at A started immediately; that at B commenced after a latent period of 18 sec. and developed and relaxed more slowly.

Repeated intraportal injections of small doses of venom into the perfused liver. With smaller doses of venom the depigmentation of the liver and the output of protein, of histamine and of the substance causing the slow contraction were less extensive. In one experiment, in which 1 mg. of cobra venom (in 0·2 c.c.) was injected into a piece of liver weighing 77 g., 22% of the liver histamine was recovered in the perfusate during 6 hr. In another, in which 51 g. of liver containing about 4·5 mg. of histamine were perfused, the injection of 0·2 mg. of cobra venom (in 0·2 c.c.) produced an output of only 40μg. of histamine. Doses of venom of this order in a larger volume, if injected for the first time, usually caused no output of histamine. But if the injection was repeated, histamine appeared in the perfusate in increasing amounts with successive injections (Fig. 12). A piece of liver weighing 70 g. and containing 36μg. of histamine per g. was perfused and four successive injections of 0·3 mg. of venom (in 1·5 c.c.) were made. In the perfusate collected after the first injection (at A) there

was no detectable amount of histamine, though it contained protein and in the sample collected during the first 5 min. some pigment was present. This sample if added to the isolated jejunum in amounts of 0·4 c.c. caused a slight slow contraction after a latent period of some 20 min. The second injection of venom given 30 min. later (at B) caused an evanescent output of 1·8μg. of histamine. The output of protein was increased and the sample collected during the first 5 min. had a brownish tint and contained small amounts of the principle that caused slow contraction of the gut. The histamine output after the third injection (at C) amounted to 5·8μg. and

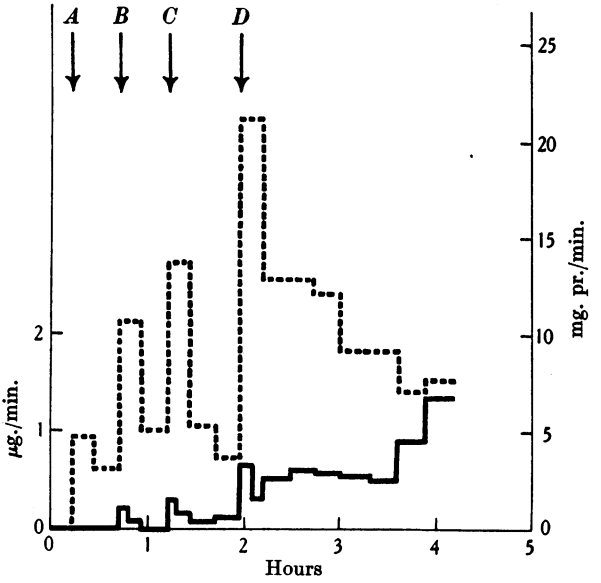

Fig. 12. Output of histamine (continuous line) and of protein (dotted line) from perfused dog's liver. At the arrow, 0·3 mg. cobra venom. Ordinates and abscissae as in Fig. 3.

was more delayed; it had not come to an end after 30 min., when the fourth dose of venom was injected. This caused an immediate output of histamine followed by a long-lasting secondary output, which was still increasing after 2½ hr. when perfusion was stopped. The total histamine assayed during this period amounted to 111μg.

Perfusion of the liver following on intravenous injection of venom. In a few experiments we have tried to ascertain whether the liver of a dog poisoned by intravenous injection of cobra venom would give off its histamine during an ensuing perfusion. A dose of 2 mg. per kg. of venom was injected intravenously into dogs under chloralose anaesthesia. 30–60 min. later, when the animals were about to die, a cannula was tied

into the portal vein and perfusion of the swollen liver commenced. The perfusate collected during the first hour was very turbid and reddish, containing protein, blood corpuscles and blood pigment. Samples tested on the gut caused a strong slow contraction but there was no indication of the presence of histamine. Later on the protein content and the reddish colour diminished and histamine appeared in increasing amounts, the histamine effect being followed by strong after-contraction. The liver gradually became somewhat depigmented, but even after several hours it showed reddish areas on section and the perfusate still contained small

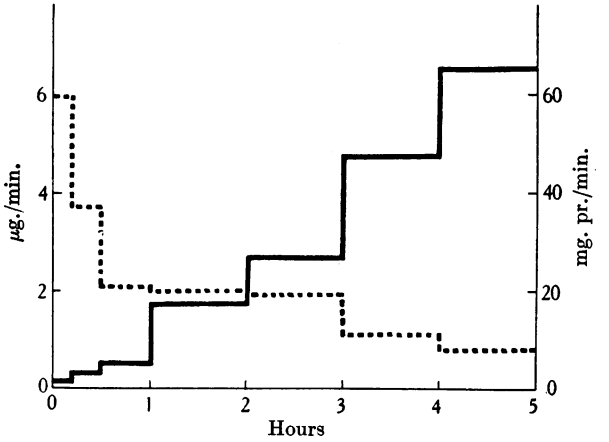

Fig. 13. Output of histamine (continuous line) and of protein (dotted line) from perfused liver of envenomed dog. Ordinates and abscissae as in Fig. 3. (Details in text.)

amounts of blood pigment. In Fig. 13 is shown the output, from the first hour onward, of histamine and of protein from a piece of liver weighing 139 g. at the end of the perfusion. Perfusion of the liver was started 30 min. after the injection of 20 mg. of cobra venom into a dog weighing 10 kg. The output of histamine during 5 hr. amounted to nearly 1 mg. which corresponded to about 20% of the available histamine. The output of protein was large, and early in the second hour it amounted to 60 mg. per min. Probably part of it was derived from plasma and blood corpuscles accumulated in the tissue spaces of the swollen liver.

E. *Monkey's liver*

The histamine equivalent per g. of liver is less than 1 μg. and generally less than 0·5 μg. After the intraportal injection of cobra venom (10 mg. or more) into the perfused liver, depigmentation occurs. If the venom is evenly injected all parts of the liver participate, otherwise those which

are not reached by the venom retain their brown colour. Liver pigments appear in the perfusate, reaching their maximum concentration during the first 10–15 min. after the injection. There is little swelling of the organ, the edges of which remain sharply defined, but the perfusate becomes cloudy and contains protein. Fig. 14 shows the output of protein per min.

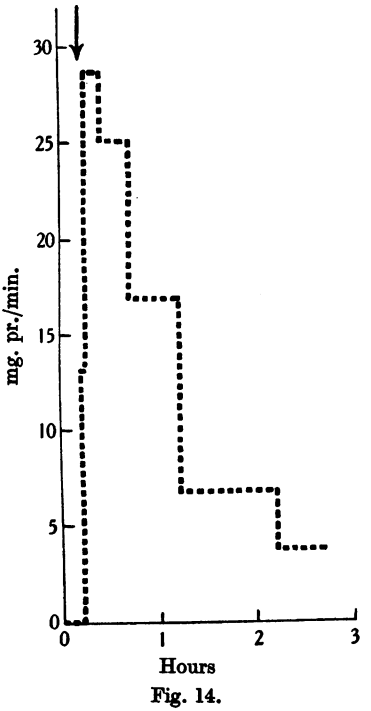

Fig. 14.

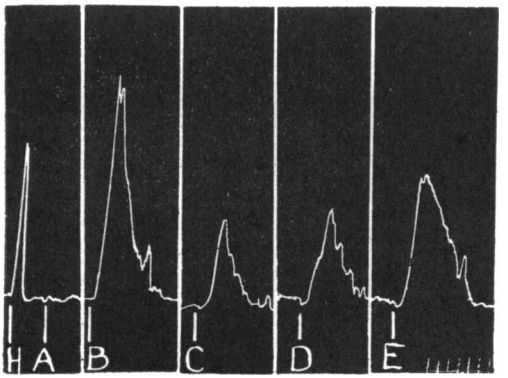

Fig. 15.

Fig. 14. Output of protein from perfused monkey's liver. At the arrow, cobra venom. Ordinates, mg. of protein per min. Abscissae, time in hours.

Fig. 15. Record from guinea-pig's jejunum desensitized to 100μg. cobra venom. At A, B and C, unboiled perfusate; at D and E, unboiled saline extracts from monkey's liver, at H, 0·04μg. histamine. Time in 30 sec. (Details in text.)

from a piece of liver weighing 73 g. after the injection of 10 mg. of cobra venom. The total output of protein was 1·6 g.

No immediate histamine-like contraction could be elicited on the guinea-pig's jejunum with perfusate before or after the injection of venom. Samples collected before had no stimulating action, and those collected after caused only delayed slow contraction. This is shown in Figs. 15 and 16. In Fig. 15, 0·2 c.c. of perfusate collected before (at A) and in the

second ½ hr. after the injection of 30 mg. of cobra venom (at B), were tested and kept in contact with the muscle for 30 sec. The rate of perfusion was 7 c.c. per min. The contraction at B commenced after a latent period of 10 sec. and was less rapid than that caused by histamine. At C, 0·02 c.c. of this sample caused a slow contraction after a latent period of 20 sec. In some experiments 0·002 c.c. of perfusate was active. If the perfusion of the liver was continued for a longer period, the activity of the perfusate gradually decreased. In Fig. 16 are shown the effects on the gut of 0·2 c.c. of perfusate collected before (at A) and in successive samples after the injection of venom, the rate of perfusion being between 6 and 7 c.c. per min. The sample tested at B was collected 5–15; that at C, 15–30; at D, 30–60; at E, 60–120; at F, 120–150 and at G, 150–170 min.

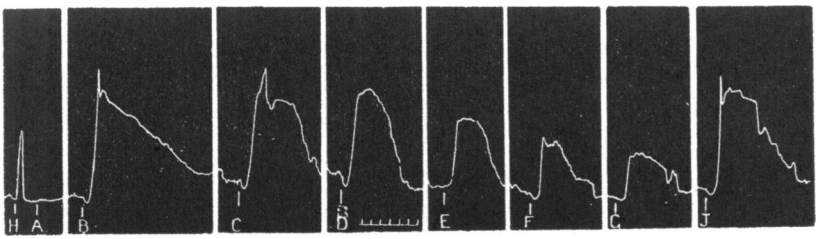

Fig. 16. Record from guinea-pig's jejunum desensitized to cobra venom. Boiled perfusate from monkey's liver before (A) and after 10 mg. cobra venom (B to J). At H, 0·04 μg. histamine. Time in 30 sec. (Details in text.)

after the injection. The samples from B to G diminished progressively in activity. There was no change in the sensitivity of the gut, which contracted strongly when the sample tested at B was retested at J. The latent period lengthened with the successive samples being less than 20 sec. at B and more than 30 sec. at G. Perfusate after boiling was less active in causing slow contraction of the gut. In Fig. 19 the effects of 0·2 c.c. of a sample of perfusate before (at A) and after boiling (at B) are compared.

After recovery from the slow contraction the reactivity of the muscle to histamine was altered in the manner already described. The response to other stimulating substances, e.g. acetylcholine, was similarly affected. When the muscle exhibited spontaneous rhythm this was often increased during increased excitability and disappeared during the period of depressed excitability, during which the muscle was in a slightly more relaxed condition. Depressed excitability is illustrated in Fig. 19A.

The substances causing slow contraction and changes in excitability of the gut must be formed by the action of the venom. A similar conclusion

was reached from the experiments on dog's organs. Saline extracts of normal monkey's liver in amounts corresponding to 1–2 mg. of tissue have no action on the gut, but in doses of 5 mg. or more cause delayed slow contraction followed by the typical changes in reactivity. This observation indicates the presence in normal liver of small quantities of a substance, which may be identical with that found in the perfusate after the injection of venom. Extracts of envenomed liver, however, were found to be 20–80 times more active, a slow contraction being produced by amounts corresponding to 0·2–0·5 mg. of liver. In Fig. 15 are seen the effects of 5 mg. of normal (at D) and of 0·5 mg. of envenomed liver (at E). Further evidence for the assumption that the substance is formed in the

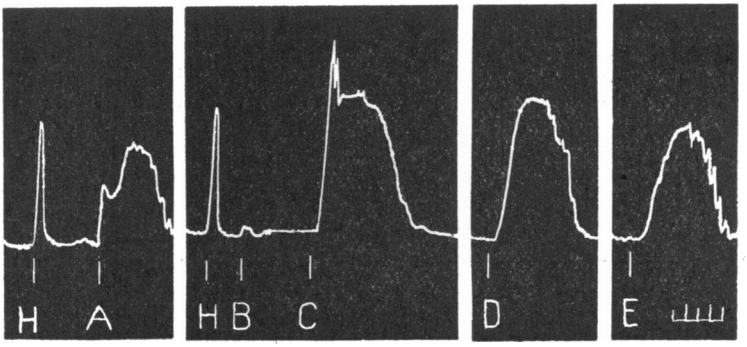

Fig. 17. Record from guinea-pig's jejunum desensitized to cobra venom. At A, 40 mg. and at B, 10 mg. normal monkey's liver extract. At C, 5 mg., at D, 1 mg. and at E, 0·5 mg. "envenomed liver" extract. At H, 0·04μg. histamine. Time in 30 sec.

tissue is given by those experiments in which the venom was not uniformly distributed throughout the organ. Extracts made from the depigmented portions were many times more active than those from regions which had not lost their pigment. Boiling reduces the activity of saline extracts to some extent, but the difference between normal and envenomed liver is still evident. The principle responsible for the slow contraction of the gut is soluble in absolute methyl alcohol, and alcoholic extracts of normal and envenomed liver exhibit similar differences in activity. In Fig. 17 alcoholic extract corresponding to 10 mg. of normal liver was without effect on the gut (at B). The histamine equivalent of this liver was less than 0·5μg. per g., but 40 mg. of liver (at A) caused an immediate histamine effect, followed by slow contraction after changing the Tyrode solution. Extracts of envenomed liver (at C, D and E) were effective in much smaller amounts.

PART 2. COMPARISON OF THE ACTIONS OF "ENVENOMED LIVER", "LYSOCITHIN" AND COBRA VENOM

The fact that extracts from envenomed liver of the monkey yielded a rich supply of the substance which causes the slow contraction of the guinea-pig's gut enabled us to use them as a source of the substance for pharmacological investigation. In preliminary experiments saline extracts were used, which were boiled and kept in the cold room for a day or two without loss of activity. The experiments recorded here were made with extracts of monkey's liver in absolute methyl alcohol in which the active principle, like lysocithin, is soluble and stable.

To test the hypothesis that lysocithin-like substances are responsible for the activity of perfusate and of extracts of envenomed liver we had first to ascertain whether the extract made by the action of cobra venom on lecithin ("lysocithin") caused similar effects on the jejunum of the guinea-pig and whether extracts of envenomed liver resembled lysocithin in haemolytic activity. We shall see that "lysocithin" and "envenomed liver" possess both these actions. These may be properties of a single substance, but they may equally well be properties of different substances which may be derivatives of lecithin or of other lipins present as impurities. Sodium oleate had no stimulating action on the guinea-pig's gut, even in amounts much greater than could have been present in our extracts of lysocithin. This excludes the possibility that oleic acid itself is responsible.

The possibility that the formation of lysocithin-like substances might be an intermediate stage in the liberation of histamine made it necessary to ascertain whether "lysocithin" itself and extracts of envenomed liver could cause the liberation of histamine from perfused organs. If these hypotheses were substantiated, the formation of lysocithin-like substances must play a significant role in poisoning by cobra venom. We have, therefore, compared some additional actions of "envenomed liver", of "lysocithin" and of cobra venom, including some which cannot be attributed to histamine.

A. *Guinea-pig's jejunum*

The actions of "envenomed liver", "lysocithin" and cobra venom on the guinea-pig's jejunum are similar. All three cause a slow contraction after a latent period of 5–30 sec., and when the muscle has recovered it may exhibit periods of increased and decreased reactivity to histamine and other stimulant substances (Fig. 19). Whereas the muscle is readily

desensitized to cobra venom by its repeated administration, such a phenomenon is not characteristic for "envenomed liver" or "lysocithin".

"*Envenomed liver.*" Fig. 17 illustrates slow contractions caused by different amounts of extracts. These contractions are usually followed by the characteristic changes in reactivity of the muscle to histamine and acetylcholine. Fig. 18 illustrates increased reactivity to histamine following the contraction caused by 1 mg. of "envenomed liver" (at A), and Fig. 19 a long-lasting period of decreased reactivity following the contraction caused by 10 mg. (at C). These results are unaffected by desensitization of the gut to cobra venom. When "envenomed liver" is given

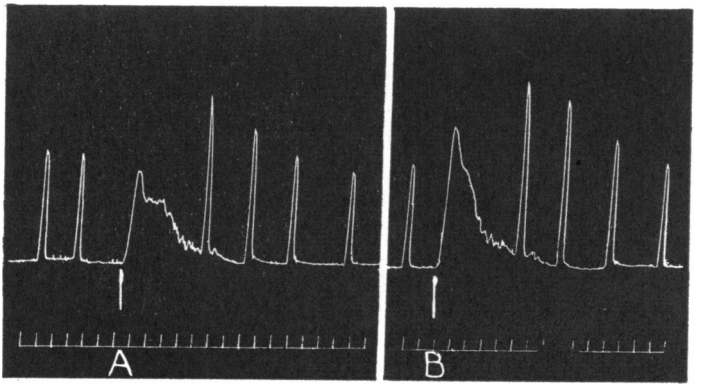

Fig. 18. Record from guinea-pig's jejunum desensitized to cobra venom. At A, 1 mg. "envenomed liver"; at B, "lysocithin" equivalent to 150μg. lecithin. The remaining responses are to 0·04μg. histamine. Time in 30 sec.

repeatedly the contractions gradually become weaker, and in some instances the muscle becomes almost insensitive to it though not to histamine. In a few experiments this condition was precipitated by cobra venom (10–40μg.) given after several doses of "envenomed liver". A similar condition was produced by repeated administration of large amounts of alcoholic extracts of normal liver (50–100 mg.).

In contrast to the behaviour of perfusates from "envenomed liver" watery solutions of the alcoholic extract do not lose activity by boiling. Nevertheless, the principle causing slow contraction in perfusates and extracts appears to be the same, since extract added to perfusate becomes less active by boiling. To two samples of 10 c.c. of the same perfusate from envenomed liver the same amount of extract was added, in one before, and in the other after, boiling. The latter mixture was more active.

The slow delayed contraction caused by perfusate or extract from envenomed liver, though closely resembling the response of the gut to cobra venom, cannot be attributed to the presence of the venom. The

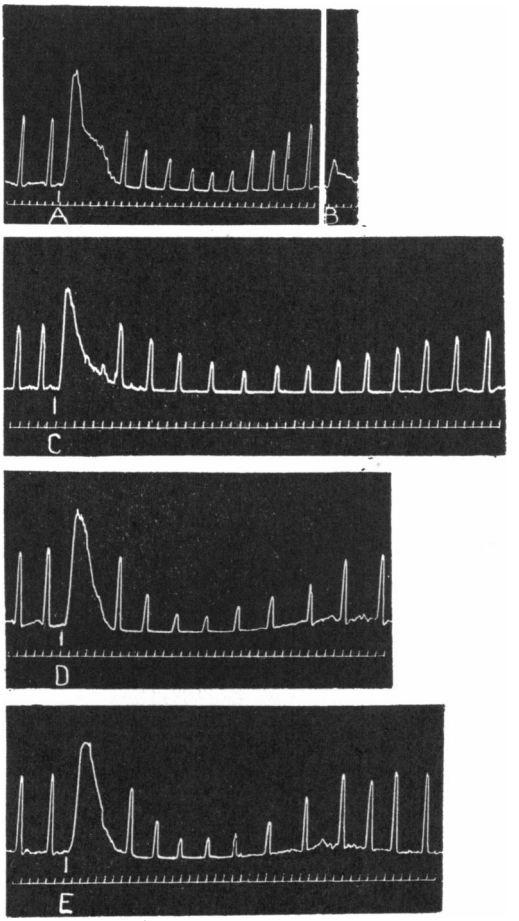

Fig. 19. Records from jejunum from three different guinea-pigs; A and B on one preparation. A and B, perfusate (0·2 c.c.) from envenomed monkey's liver; A, unboiled; B, boiled. At C, 10 mg. "envenomed liver". D and E on one preparation; at D, "lysocithin" equivalent to 200 μg. lecithin; at E, 30 μg. cobra venom. Doses left in contact for 30 sec. The remaining responses are to 0·04 μg. histamine. Time in 30 sec.

contractions were usually produced on a gut desensitized to 50 or 100 μg. of venom, so that to be effective venom must have been present in amounts larger than these. This was not possible. The contractions shown in Fig. 16 were produced by different samples of 0·2 c.c. of perfusate the

total amount of which exceeded 1 litre. Since in this experiment 10 mg. of venom had been injected the amount present in 0·2 c.c. must have been less than 2 μg. The alcoholic extract of the 60 g. of liver at the end of the perfusion was active in a dose of 0·5 mg., and even if all the venom were present in the liver and it were soluble in alcohol, which is not the case, this dose could not contain more than 0·1 μg. Finally, repeated administration of perfusate or extract from envenomed liver did not desensitize the gut to small doses of cobra venom.

"*Lysocithin.*" Small amounts of "lysocithin" cause a delayed slow contraction of the gut (Figs. 18B, 19D and 20). The sensitivity of preparations of jejunum from different guinea-pigs varied greatly, independently of their reactivity to histamine. On some, "lysocithin" equivalent to 4 μg. of lecithin caused a strong reaction, on others equally sensitive to histamine 10–30 times this dose was required for a similar response. There was usually a close resemblance between these effects and those produced by "envenomed liver" on the same preparation, though sometimes relaxation of the muscle was slower after "envenomed liver" than after "lysocithin". The muscle relaxed gradually when the "lysocithin" was washed away and exhibited the characteristic changes in reactivity to stimulant substances. Increased reactivity to histamine is illustrated in Fig. 18B and decreased reactivity in Fig. 19D. The "lysocithin" contractions in Figs. 18 and 19 started after a latent period of 15 sec. In Fig. 18 the change in excitability is compared on the same preparation with that following a corresponding contraction caused by "envenomed liver" (at A) and in Fig. 19 with that following a contraction by cobra venom (at E). In those preparations in which the contraction caused by "lysocithin" was not followed by changes in reactivity of the muscle to histamine, such changes were absent after the contraction caused by "envenomed liver" or by cobra venom.

The contraction caused by "lysocithin" closely resembles that produced by cobra venom, but the method, by which "lysocithin" was prepared, excluded the presence of venom in the final methyl alcohol extract and the contraction was usually not influenced by desensitization to cobra venom (Fig. 20). At A, D and E the same amounts of "lysocithin" were added to the bath. B shows the effect of 10 μg. of cobra venom, a second dose of venom given at C was ineffective, but the muscle still reacted to "lysocithin". Between D and E three doses of 100 μg. of cobra venom were given, the first and second caused slow and weak contractions, the third had no effect. Though the muscle was desensitized to doses of venom of this order, its reactivity to "lysocithin" was not materially altered.

The effect of "lysocithin" could be obtained repeatedly on the same preparation but the contractions gradually became weaker and sometimes were further reduced by cobra venom. The reaction to histamine under these conditions remained unchanged or increased. We have described the same course of events after repeated doses of "envenomed liver".

Lysocithin is resistant to heat and the preparation of our alcoholic extracts involved heating for considerable periods. Boiling a watery solution of "lysocithin" did not diminish its activity, but when it was added to perfusate from envenomed monkey's liver and brought to the boiling point its activity was reduced like that of "envenomed liver".

In a few experiments we have examined the influence of alcoholic extract from normal monkey's liver on the action of "lysocithin". Alcoholic extracts of "lysocithin" and of normal liver were evaporated together and taken up in Tyrode solution. It was found that extracts of a few milligrammes of normal liver did not diminish the contraction caused by "lysocithin", though a slightly longer latent period suggested some inhibitory influence.

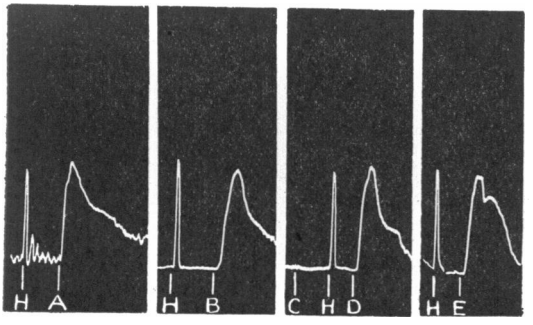

Fig. 20.

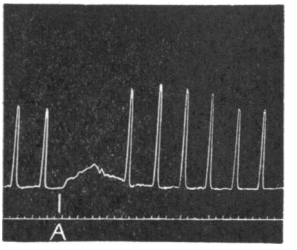

Fig. 21.

Fig. 20. Record from guinea-pig's jejunum. At A, D and E, "lysocithin"; at B and C, 10 μg. cobra venom. At H, 0·04 μg. histamine. (Details in text.)

Fig. 21. Record from guinea-pig's jejunum. At A, third administration of 10 μg. cobra venom. The remaining responses are to 0·02 μg. acetylcholine. Time in 30 sec.

Cobra venom. In addition to its stimulating action venom causes the same changes in reactivity as described for "envenomed liver" and for "lysocithin". The strong contraction of a first dose of venom is often followed by decreased reactivity, whereas a slight stimulating effect produced on the partly desensitized muscle by the same dose of venom applied for the second or third time is followed by increased reactivity,

this dose of venom produces no changes of reactivity when the muscle is completely desensitized. This indicates that the changed reactivity results from an indirect action of the venom and it is reasonable to assume that the formation of lysocithin-like substances in the muscle is responsible.

Fig. 19 illustrates decreased reactivity to histamine after a contraction caused by 30μg. of venom (at E). The muscle had previously been subjected to 10μg. of venom but was only partly desensitized. The contraction started after a latent period of 12 sec. The muscle exhibited small spontaneous movements which disappeared during the period of decreased reactivity. In Fig. 21 is shown the effect of a third dose of 10μg. of cobra venom on another preparation (at A). The weak contraction is followed by a short period of increased reactivity to acetylcholine.

B. *Guinea-pig's uterus*

The uterus of the guinea-pig responds by contraction to cobra venom but is easily desensitized. "Lysocithin" and the active substances in perfusate and extract of envenomed liver also cause contraction which is not affected by previous desensitization with venom and can be repeatedly elicited without appreciable diminution. Fig. 22C illustrates a contraction produced (at I) by "lysocithin" equivalent to 2 mg. of lecithin and Fig. 22D a contraction (at I) caused by 10 mg. of "envenomed liver".

C. *Guinea-pig's uterus poisoned by histamine*

Schild [1936] has shown that the uterus poisoned by large doses of histamine responds to the addition of further histamine by relaxation but that the anaphylactic contraction can still be elicited. He concludes "that either the histamine released from the cells has a different action from that of histamine applied to the cell outer surface or it plays only a secondary part in anaphylactic shock".

The uterus poisoned by histamine still contracts to cobra venom "lysocithin" and "envenomed liver" though the contractions have a longer latent period and are more gradual and less in extent than those of the normal uterus. Fig. 22B illustrates the slow contraction (at I) of the poisoned uterus to a first dose of 20μg. of cobra venom. The contraction caused by such a dose on a normal uterus is stronger and quicker. Fig. 22C shows that the "lysocithin" contraction of the poisoned uterus (II) is slower and weaker than that caused on the normal uterus (at I). The latent period at I was 4 sec., that at II, 30 sec. At II in Fig. 22D is shown the contraction of the poisoned uterus caused by 40 mg. of

"envenomed liver". It started after a latent period of 18 sec. The contraction of the normal uterus (at I) was caused by 10 mg. of "envenomed liver" and commenced after a latent period of 8 sec.

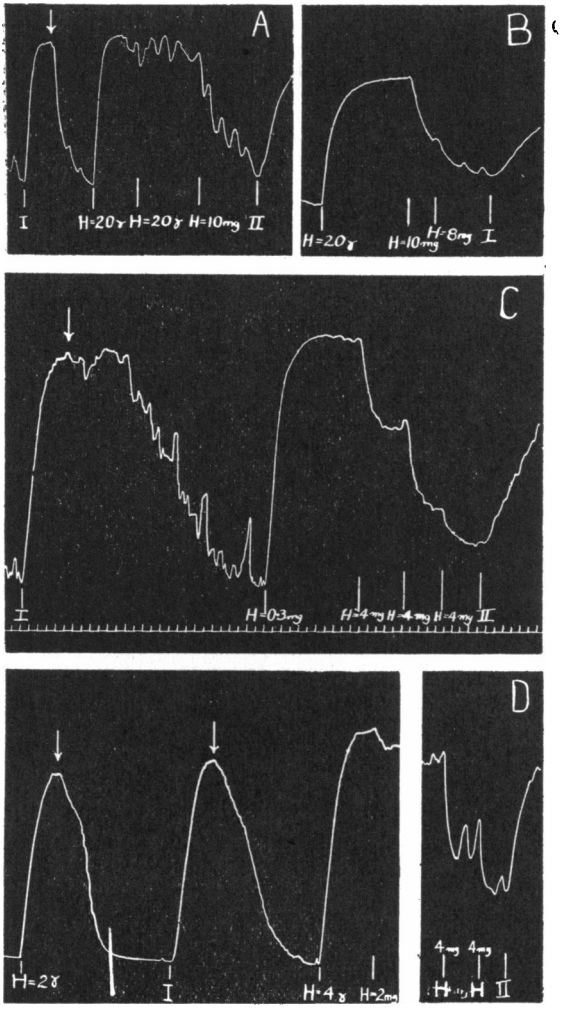

Fig. 22. Records from the uteri of four virgin guinea-pigs. A, at I and II, 10 mg. peptone (Berner). B, at I, 20 μg. cobra venom. C, at I and II, "lysocithin". D, at I and II, "envenomed liver". At H, histamine in various amounts. At the arrows, the bath washed out. Time in 30 sec. (Details in text.)

It was of interest to extend these observations to another cell-injurious agent—peptone, which like "lysocithin" and "envenomed liver" has the advantage that several doses can be given to the normal uterus without

appreciable reduction in the extent of the contraction and that comparison of the effects before and after poisoning can be made on the same horn of the uterus. Such an experiment is illustrated in Fig. 22 A, which shows the effect of 10 mg. of peptone before (at I) and during histamine poisoning (at II). The contraction at II is weaker, more gradual and starts after a longer latent interval than that at I.

D. *Rat jejunum*

The jejunum of the rat is insensitive to histamine but responds by contraction to concentrations of 1 : 40,000 to 1 : 50,000. It also contracts with cobra venom, "envenomed liver" and "lysocithin", but individual variation in sensitivity is great and a few preparations did not respond to any of the three substances though they reacted well to

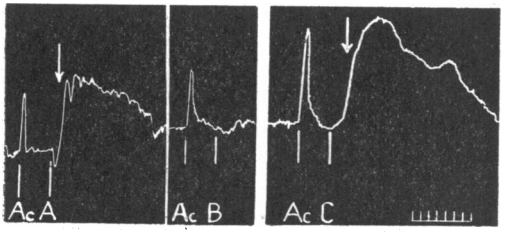

Fig. 23. Records from jejunum of two rats. At A and B, 10 μg. cobra venom on same gut; at C, 5 mg. "envenomed liver". At Ac, 0·08 μg acetylcholine. At the arrows, bath washed out. Time in 30 sec.

acetylcholine. The gut is readily desensitized to cobra venom, but this has little effect on its reaction to "envenomed liver" or "lysocithin". By repeated administration of "envenomed liver" or of "lysocithin" the contractions usually diminish, and sometimes fail to occur. Fig. 23 shows the effect of two successive doses of 10 μg. of cobra venom (at A and B) and on another gut of 5 mg. of "envenomed liver" (at C). The contractions start after a latent period of about 20 sec., the muscle continues to contract after changing the Tyrode solution and only relaxes slowly.

E. *Cat's heart*

"Envenomed liver" in contrast to "lysocithin" has a relatively feeble action on the cat's heart. This discrepancy may be accounted for by the presence in liver extracts of substances which antagonize the toxic action of "lysocithin" on the heart. The action of "lysocithin" is not wholly comparable to that of cobra venom.

"*Envenomed liver.*" Filtered extracts cause acute changes in the coronary flow and have a more gradual effect upon the heart beat. A first dose causes dilatation of the coronary vessels which lasts from 4 to 7 min., but no dilatation occurs following subsequent doses. In the course of 2 hr. the heart beats decrease in force without significant changes in rate, and a gradual diminution in coronary flow occurs. If the heart does not recover it becomes oedematous and irregularities of conduction ensue. It finally comes to rest in diastole or in the mid-position but not in systole. The left ventricle first ceases to beat, then the right ventricle and finally the auricles. In the experiment of Fig. 24, 1 g. of "envenomed liver" and 1 hr. later 2 g. were injected. The first injection (at I) produced an in-

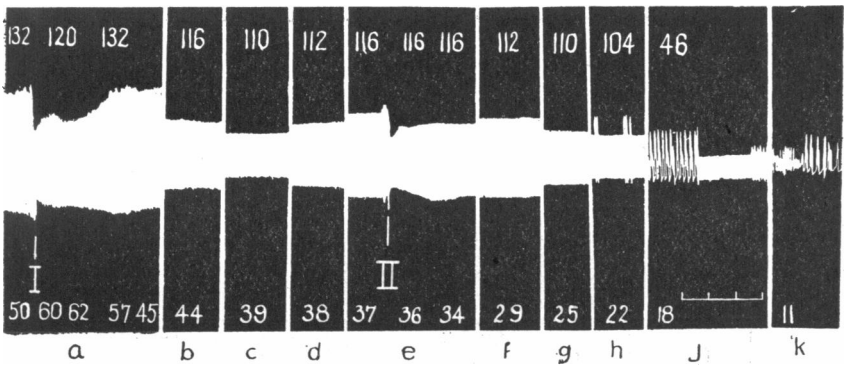

Fig. 24. Record from isolated cat's heart. At I, 1 g., at II, 2 g. "envenomed liver". In this and the following three figures the numbers above are heart rate per min.; below, coronary outflow in c.c. per min. *b* to *e*, 20, 30, 50 and 60 min. after I; *f* to *k*, 15, 30, 40, 60 and 80 min. after II. Time in 30 sec.

crease in coronary flow from 50 to 62 c.c. per min.; the flow then decreased continuously. The force of the heart beats diminished gradually but some recovery occurred. The rate diminished only slightly. After the second injection (at II) there was no coronary dilatation, the force of the heart beats decreased and extra-systoles appeared within 40 min. (at *h*) leading to heart block within an hour (at *j*). Eventually (at *k*) the whole heart stopped in diastole for periods of 5–10 sec., between which there were irregular groups of contractions of the right heart. Later on the periods of diastolic standstill became longer. Equivalent amounts of extracts from normal monkey's liver were without effect.

"*Lysocithin.*" We used a batch of "lysocithin" which, in a dose equivalent to 1 mg. of lecithin, corresponded in its action on the jejunum of the guinea-pig to 5 mg. of the preparation of "envenomed liver" used

in the heart experiments. The effects of "lysocithin" in a dose equivalent to 1 mg. of lecithin are shown in Fig. 25 (at I). There is vasodilatation lasting 6 or 7 min., which is preceded during the first minute by decrease

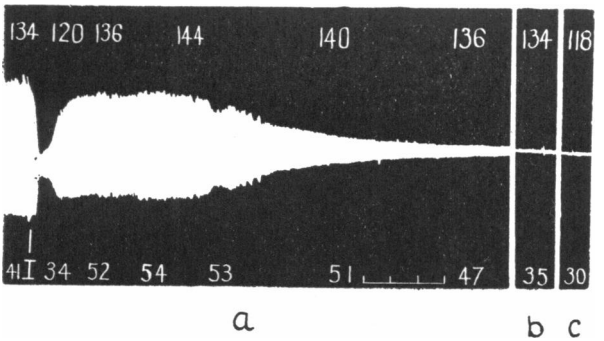

Fig. 25. Record from isolated cat's heart. At I, "lysocithin". *b* is 30, *c* 80 min. after *a*. Time in 30 sec. (Details in text.)

in coronary flow. Later the flow gradually decreases. The diminution in extent of the heart beat proceeds rapidly. If repeated small doses of "lysocithin" are given, the early vasodilatation occurs only after the first injection. Doses of "lysocithin" equivalent to 5–10 mg. of lecithin

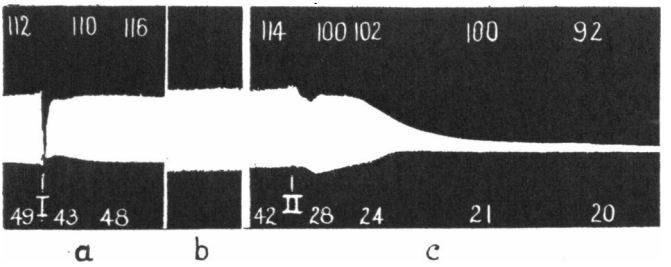

Fig. 26. Record of isolated cat's heart. At I, "lysocithin" with extract of normal liver; at II, "lysocithin" alone. (Details in text.)

cause immediate vasoconstriction of the coronary vessels which is not followed by dilatation. The extent of the beat and the coronary flow progressively diminish, the heart rate is slowed, irregularities of conduction occur and eventually the heart ceases to beat in diastole or in the mid-position, but not in systole. The left ventricle stops first, then the right ventricle and finally the auricles.

In several experiments we have injected different amounts of "lysocithin" with the extract of 1 g. of normal monkey's liver. With 5 and

10 mg. of "lysocithin" the mixtures were without significant effect. If larger amounts of "lysocithin" were injected with this amount of normal liver the changes resembled those produced by the injection of "envenomed liver". The protective action of normal liver extract is illustrated in Fig. 26. "Lysocithin" equivalent to 10 mg. of lecithin, injected at I, together with extract of 1 g. of normal liver produced no obvious changes on the heart beat and only a trivial decrease in coronary flow. Half the amount of "lysocithin" injected alone at II caused rapid failure of the heart, with decrease in coronary flow.

Cobra venom. Unlike many previous workers who have studied the effects of continuous perfusion with various concentrations of cobra venom upon the isolated heart, we have investigated the effects of single injections of venom which pass rapidly through the heart. The injection of 2–4 mg. of venom usually causes systolic contracture of the heart within a few minutes (Fig. 27). The systolic contracture is preceded by a decrease in the extent of the force of the heart beat and by a slight increase in its rate. The coronary flow within a minute after the injection increases nearly twofold and remains rapid though the heart is in systolic contracture. With smaller doses of venom (1 mg.) the changes in the heart beat proceed more gradually. There is diminution in the extent of the heart beat and after some 40–50 min. extra systoles or short periods of heart block occur; but in spite of these the heart is still recording 1½–2 hr. after the injection. If the coronary flow is large (70–80 c.c. per min.) before the injection there is some decrease in flow in the first 1 or 2 min. after the injection; an initial moderate flow is increased by about 50% during the first few minutes. Extracts of normal monkey's liver appear to exert some protective influence against the toxic action of cobra venom.

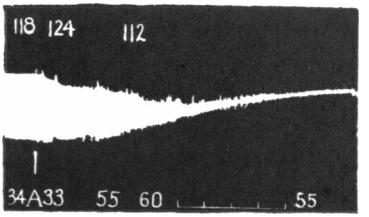

Fig. 27. Record of isolated cat's heart. At A, 2 mg. cobra venom. Time in 30 sec.

F. *Rabbit's eye*

"Envenomed liver", "lysocithin" and cobra venom when injected into the anterior chamber of the eye cause closely similar and characteristic changes. These have already begun within 4 hr. after the injection and reach their full development in 24 hr. The eye appears to bulge from the socket. The cornea becomes cloudy and so opaque that the pupil can no longer be seen; there is an associated conjunctivitis. Fig. 28 illustrates

these changes by photographs: A, 24 hr. after 0·4 g. of "envenomed liver"; B, 24 hr. after "lysocithin" equivalent to 3 mg. of lecithin; and C, 7 days after the injection of 2 mg. of cobra venom. The injections were made in a volume of 0·1 c.c. These changes did not clear up in the course of 2 months. Doses, a tenth to a quarter as large, produced slight and transient congestion of the iris without gross corneal changes. Within 48 hr. the injected eye could not be distinguished from its untreated fellow.

Extract of normal liver in large amounts had no such action. Fig. 28D is a photograph 24 hr. after the admixture of 0·8 g. of normal monkey's liver with the contents of the anterior chamber. The suspended material had been absorbed and the eye was almost normal.

G. *Intravenous injection in guinea-pigs*

The injection of "envenomed liver" and of "lysocithin" into guinea-pigs by a jugular vein, exposed under local anaesthesia, caused similar symptoms starting after a latent interval of 5–15 sec. There was cough and obstructive dyspnoea, and lethal doses caused death from asphyxia. Cyanosis of the ears and convulsions were predominant features, and death was preceded by coma with evacuation of the bladder and the passage of a few faecal pellets. In animals which recovered after severe symptoms, instead of a few terminal and ineffective respirations, the respiratory rate suddenly quickened, the bronchial constriction was overcome, convulsions, if present, ceased, the corneal reflex returned and after a period of collapse the animals were restored almost to normal. These are the symptoms of acute anaphylactic shock in guinea-pigs. In a few animals there was an additional symptom which is never present in acute anaphylaxis—blood-stained frothing from the nostrils, the manifestation during life of acute oedema of the lungs. A similar symptom has been observed by Belfanti [1925] after the intravenous injection of lysocithin into rabbits. There is yet another respect in which the picture differs from acute anaphylaxis. The administration of a sublethal dose of "envenomed liver" or of "lysocithin" fails to protect the animal from a lethal dose of either injected shortly afterwards. In some of the guinea-pigs, in which "envenomed liver" was injected, collapse, lasting 4–10 min., was the dominant feature. Post-mortem the lungs were distended and did not collapse when the thorax was opened, but, on section, oedema and a few haemorrhages were invariably present. The heart was generally beating feebly, the right chambers being full of dark blood. Extracts of normal monkey's liver in dosage equivalent to twice the lethal dose of "envenomed liver" caused no symptoms when injected intravenously.

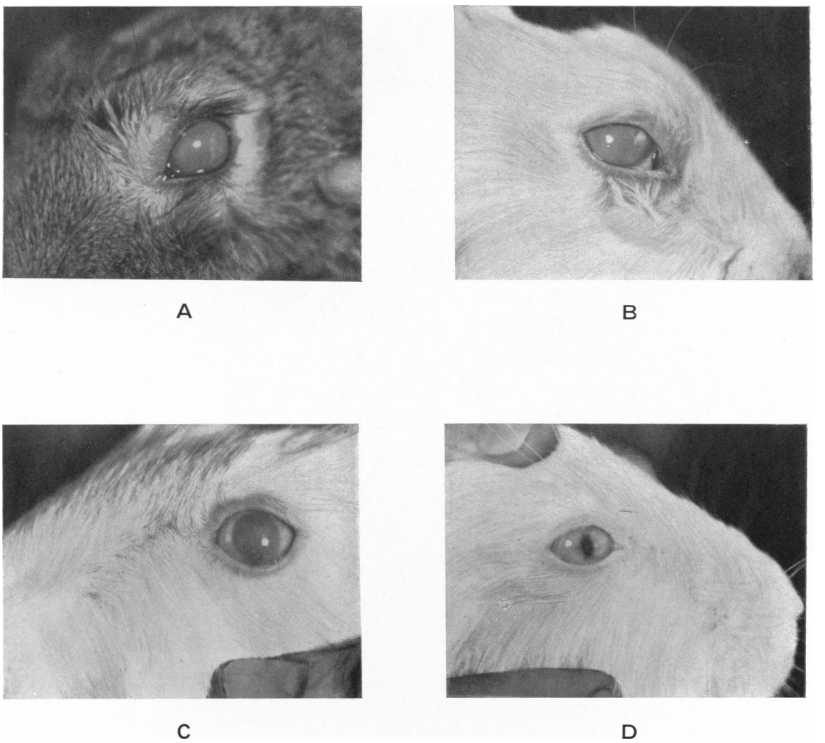

Fig. 28. Photographs of rabbits' eyes after injections into anterior chamber. A, of "envenomed liver"; B, of "lysocithin"; C, of cobra venom, and D, of normal liver extract. (Details in text.)

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The toxicity of "envenomed liver" and that of "lysocithin" were paralleled by their haemolytic action rather than by their stimulant effect upon the isolated jejunum of the guinea-pig. Tables I and II give the

TABLE I. Intravenous injection of "envenomed liver" in guinea-pigs

Weight in g.	Dose in g.	Result
280	1·6	+ in 1½ min.
330	1·2	+ in 2½ min.
245	1·2	+ in 2 min.
258	1·2	+ in 1 min. 40 sec.
305	0·8	Severe symptoms during 11 min. Recovery
325	0·8	Severe symptoms during 5 min. Recovery

TABLE II. Intravenous injection of "lysocithin" in guinea-pigs

Weight in g.	Dose in mg. as lecithin equivalent	Result
250	15	+ in 2½ min.
240	12	+ in 1 min. 55 sec.
270	12	+ in 2¼ min.
300	12	+ in 1 min. 40 sec.
296	9	Severe symptoms for 5 min. Recovery
265	9	Slight symptoms. Recovery
270	7·5	No symptoms

results of titrations of the toxicity of an extract of envenomed liver and of a batch of "lysocithin". "Envenomed liver" in a dose of 1 mg. corresponded in toxicity to "lysocithin" equivalent to about 10 μg. of lecithin. On washed sheep's corpuscles the haemolytic activity of 1 mg. of this liver extract corresponded to "lysocithin" equivalent to about 15 μg. of lecithin.

H. *Sheep red blood corpuscle*

"Envenomed liver" has an immediate haemolytic action on washed sheep's corpuscles resembling that of "lysocithin" (Table III). This action is not impaired by boiling (Table IV). Extracts of normal liver and cobra venom are without action on these cells. We tested cobra venom in concentrations up to 20 mg. per c.c.

TABLE III. Haemolysis times in minutes of washed sheep's red blood corpuscles

		Dilutions						
	Undiluted	½	¼	⅛	1/16	1/32	1/64	1/128
"Envenomed liver" (0·5 g./c.c.)	2*	2*	2*	2	2	2½	8	—
"Lysocithin" (equivalent to 32 mg. lecithin/c.c.)	½	½	½	½	½	1	2	—

* It was difficult to decide the exact moment of complete haemolysis owing to the opacity of these strong dilutions. Haemolysis probably took place more quickly than is indicated by these times.

When the haemolytic and muscle stimulant activities of "envenomed liver" and of "lysocithin" were compared there was no quantitative agreement. Envenomed liver extracts were proportionately more active upon the gut than by tests of haemolysis. The extract used in the experiments of Table IV in an amount equivalent to 1 mg. of liver had about

TABLE IV. Haemolysis times in minutes of washed sheep's red blood corpuscles

		Dilutions					
	Undiluted	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$
"Lysocithin"	1	1	$1\frac{1}{2}$	3	10	87	—
"Lysocithin" heated to boiling	1	$1\frac{1}{2}$	$2\frac{1}{2}$	3	11	90	—
"Lysocithin" with normal liver (1 g./c.c.)	—	—	—	—	—	—	—
"Lysocithin" with normal liver (0·1 g./c.c.)	1	2	$4\frac{1}{2}$	15	51	90	—
"Envenomed liver" (1 g./c.c.)	3*	3*	3*	3	3	3	10
"Envenomed liver" boiled (1 g./c.c.)	3*	3*	3*	3	3	$4\frac{1}{2}$	12

* It was difficult to decide the exact moment of complete haemolysis owing to the opacity of these strong dilutions. Haemolysis probably took place more quickly than is indicated by these times.

the same muscle stimulant action as the "lysocithin" in an amount equivalent to 0·3 mg. of lecithin, but compared by haemolysis corresponded with "lysocithin" equivalent to 0·03 mg. of lecithin. This discrepancy may be accounted for by the antihaemolytic action of an alcohol soluble substance or substances present in liver, since normal liver extract exerts a protective effect (Table IV). This, however, does not wholly account for the weaker haemolytic action of "envenomed liver". In one experiment, for instance, in which 1 mg. of "envenomed liver" had approximately the same muscle stimulating action as "lysocithin" equivalent to 200μg. of lecithin, its haemolytic action corresponded to one-quarter of this amount of "lysocithin", even though the "lysocithin" had been tested on the red cells after admixture with extract of normal monkey's liver in amounts equal to those used for testing the haemolytic action of "envenomed liver".

I. *The perfused liver of the dog. Liberation of histamine*

The injection into the perfused dog's liver of "lysocithin" equivalent to 40–100 mg. of lecithin or of 5–10 g. of "envenomed liver" causes the release of liver pigments, of protein and of histamine and the appearance of these substances in the venous perfusate. The results resemble those obtained by repeated injections of small doses of cobra venom. A first injection of "lysocithin" or of "envenomed liver" was not always followed by the appearance of histamine in the perfusate. In some experiments it produced a histamine output of 40–50μg. and corresponding to more than 1% of the histamine available in the perfused piece of liver;

usually the amounts released were smaller. With successive injections the histamine output increased and after later injections there was an immediate output followed by a gradual and long continued secondary output.

In the experiment of Fig. 29 about 80 g. of liver containing 32μg. of histamine per g. were perfused and eight injections of "lysocithin" were made. The histamine output, which was only trivial after the first injection, increased and became more prolonged after each successive injection. The total amounts assayed were 0·75, 4·3, 13·5, 30·8, 31·7, 42·5 and 75·2μg. After the fourth injection the perfusate, 1 hr. after the injection, still contained histamine and after later injections there was a gradual secondary rise in histamine output. The output of protein increased after the second and third injections and then diminished despite the increase in

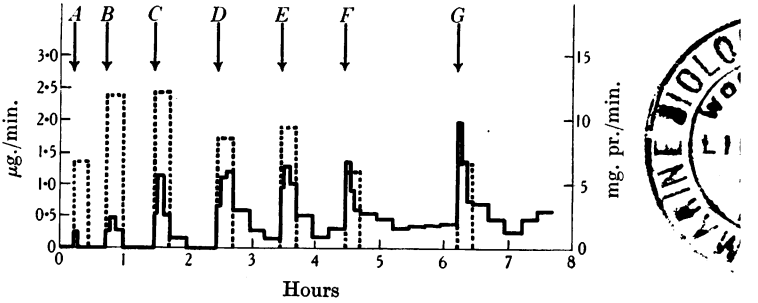

Fig. 29. Output of histamine (continuous line) and of protein (dotted line) from perfused dog's liver. At the arrows, injections of "lysocithin". In this and the following figure the protein output was determined only for the first 15 min. after each injection. Ordinates and abscissae as in Fig. 3. (Details in text.)

the output of histamine. The samples collected during the first 10 min. after each injection contained liver pigments. The perfusate collected in the first 3–4 min. after each injection caused slight after-contraction on the guinea-pig's gut, probably from the presence of part of the injected "lysocithin", most of which appeared to be destroyed or absorbed during passage through the liver. The later samples of perfusate caused no after-contraction.

"Lysocithin" and "envenomed liver", in doses having the same hæmolytic power, had approximately the same potency for the liberation of histamine (Fig. 30). A piece of dog's liver weighing 80 g. and containing 63μg. of histamine per g. was perfused. At A, C and E "lysocithin" equivalent to 45 mg. of lecithin, and at B, D and F 8·5 g. of "envenomed liver" were injected. The doses of "lysocithin" and "envenomed liver" corresponded in haemolytic power and, as the figure shows, their power of liberating histamine was of the same order. The amounts of histamine

in the perfusate after the successive injections were 0·86, 1·6, 11·1, 42·2, 76·7 and 200·5 μg.

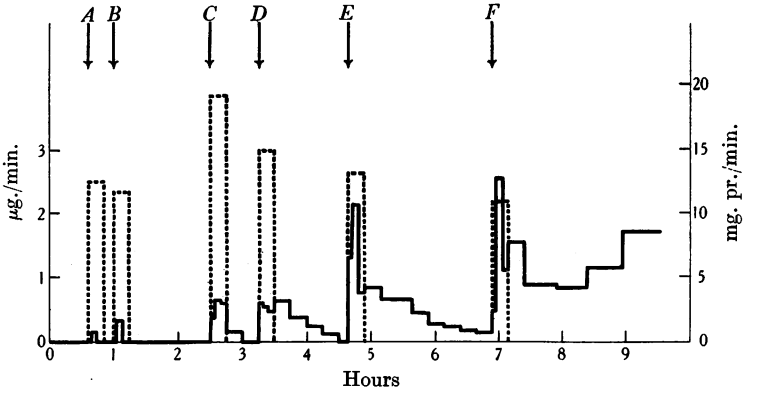

Fig. 30. Output of histamine (continuous line) and of protein (dotted line) from perfused dog's liver. At the arrows, alternate injections of "lysocithin" (A, C, E) and "envenomed liver" (B, D, F). Ordinates and abscissae as in Fig. 3. (Details in text.)

Discussion

The liberation of histamine by cobra venom which we have recently demonstrated in experiments on perfused lungs of guinea-pigs and cats has been confirmed on perfused lungs of dogs and monkeys and on the perfused liver of the dog. Apart from the presence of histamine in the perfusates from envenomed organs, our main interest was concentrated on the appearance of a substance or substances which caused slow delayed contraction of the guinea-pig's gut and unlike histamine must have been formed by the action of the venom in the tissues. The active substance or substances could be extracted with methyl alcohol from the envenomed liver of the monkey and the actions of such extracts were compared with those produced by an alcoholic extract of lecithin treated with cobra venom ("lysocithin"). Both extracts had similar effects on widely different tissues, but there were some quantitative differences. "Envenomed liver" was found to be less active in causing haemolysis and more active in stimulating the gut than was "lysocithin". This difference is only accounted for in part by antihaemolytic substances in liver extracts and makes it uncertain whether the muscle stimulating and haemolytic properties belong to one substance or to two or more. It is even uncertain whether pure lysocithin has any muscle stimulating action. Part at least of the haemolytic activity of both extracts must be attributed to lysocithin and the quantitative agreement between the extracts in haemolytic power, in ability to liberate histamine, and in toxicity for the guinea-pig suggests

that all these actions result from the presence of lysocithin. Since the active substances in both extracts appear to be formed by the action of venom on lipins, we shall provisionally regard their total activity as being due to lysocithin-like substances.

In previous communications we have compared the circulatory effects caused by intravenous injection of venom into cats [1937*c*] and into dogs [1937*d*] with those caused by histamine. We found that many symptoms were similar, and since the venom was shown to liberate histamine we concluded that these symptoms could be explained by the action of the liberated histamine. We are now confronted with the fact that venom also causes the formation in the tissues of lysocithin-like substances. Their formation probably represents an intermediate step in the liberation of histamine and precedes it, a view which is supported by the observation that doses of venom too small to cause the output of detectable amounts of histamine cause the formation of small amounts of lysocithin-like substances.

It is often stated that the liberation of histamine represents the first defence mechanism of the cell to injury and that with more intensive damage direct effects of cell injury may dominate the picture. This view has been extended to the cytolytic action of cobra venom. The so-called "direct" effects of the venom may result in part from the action of lysocithin-like substances formed in the tissues. Many effects of cobra venom which cannot be reproduced by histamine may be explained by the action of these substances, which may even contribute directly to the "histamine-like" effects of the venom. We must take into account the fact that these substances act mainly at the site of their formation, whereas when injected they have general effects. This difference may explain some of those actions of "lysocithin" and of "envenomed liver" which are not reproduced by the venom and *vice versa*.

The formation of lysocithin-like substances in the tissues is not confined to cobra venom but plays a part in the symptomatology of poisoning by other snake venoms. Perfusates collected from lungs after the injection of the venom of *Crotalus atrox* and of the Australian copperhead (*Denisonia superba*) contained a substance causing slow contraction of the guinea-pig's gut [Feldberg & Kellaway, 1937*a*], and recently Trethewie has shown that the same is true of the perfusate collected from dog's lung and liver after the injection of the venoms of the Australian black snake (*Pseudechis porphyriacus*) and death adder (*Acanthophis antarcticus*), and that extracts of the envenomed organs are powerfully haemolytic. The formation of lysocithin-like substances in the tissues also occurs with other venoms. The observation of Carpi &

Morgenroth [1906] that the action of bee venom on lecithin leads to the formation of a substance which we can regard as lysocithin, has been repeatedly confirmed, and we have shown [1937*b*] that perfusate collected from a dog's liver, after the injection of bee venom, contains in large amounts a substance (or substances) which causes slow contraction and transient changes in the reactivity of the guinea-pig's gut.

Other haemolytic poisons may act like snake venoms on the lipins of the tissues. Neuberg & Rosenberg [1907] showed that enzymes acting on lecithin are present in ricin and crotin. Neuberg & Reicher [1907] found that cholera and staphylococcal haemolysin act on lecithin, and we have recently shown that an alcohol soluble substance, which causes slow contraction and after-changes in excitability of the gut, is found in the perfusate of the perfused lungs after the injection of staphylococcal toxin. It is likely that other haemolytic bacterial toxins also form lysocithin-like substances from the tissue lipins.

We must consider the further possibility that this mechanism is not confined to the action of haemolytic poisons but plays a part in less extensive cell injury, such as is brought about by the antigen-antibody reaction of anaphylaxis, by peptone, by ultraviolet light and in short in all those reactions which have been studied on the blood vessels of the human skin by Sir Thomas Lewis and have been attributed to the liberation of *H*-substance. The formation of lysocithin-like substances from tissue lipins in such forms of cell injury would not necessarily be achieved by the direct action of the injurious stimulus but might be brought about by an enzyme set free in the tissue cells. Lysocithin-like substances appear to be easily formed in tissues. Bergenhem & Fåhraeus [1936] have shown that in blood or serum stored at 39° C. a substance appears which they regard as lysocithin formed by lecithinase. These experiments have been confirmed by Bogaert [1937]. Belfanti [1924] showed that pancreatic extracts contain a haemolytic substance which is identical with lysocithin from egg lecithin, and we have found traces of slowly contracting muscle stimulant substance in extracts of normal liver and lungs. This may be an indication that the formation of such substances plays a role in normal tissue activities. There is at present no direct evidence for this more general application of our theory, but there are a few indications in its favour. There are some symptoms common in cell injury produced by non-haemolytic poisons which cannot be explained by liberation of histamine since they are not reproduced by it. We have already mentioned one instance—the reaction of the guinea-pig's uterus poisoned by histamine. The uterus either fails to contract with histamine or actually

relaxes, but in this condition it still contracts, not only to cobra venom and to "envenomed liver", but also to peptone and, as Schild [1936] has shown, to the anaphylactic antigen. If we can assume that cell injury is associated with the formation of lysocithin-like substances from tissue lipins many of the inherent difficulties of the histamine theory of cell injury will be removed. The possibility that the muscle stimulating and haemolytic actions of lipin cleavage products are not necessarily associated is favourable to the more general application of this theory of cell injury.

Conclusions

1. The injection of cobra venom (2–20 mg.) into perfused organs (lung, liver) of dogs and monkeys causes the appearance in the venous perfusate of histamine, of protein and of a substance or substances which cause slow contraction and transient changes in the excitability of the guinea-pig's gut. In the case of the liver, pigments are also set free. No histamine appears in the perfusate of envenomed monkey's liver, since this organ has a very low histamine content. The changes in the venous perfusate from the liver of dogs poisoned by intravenous injections of cobra venom are similar to those observed when the venom is injected into the isolated organ.

2. Histamine, protein and liver pigments are *liberated* from the cells of perfused organs, but the substance (or substances) which causes slow contraction of the gut and subsequent changes in its reactivity is *formed* in the organs by the action of the venom.

3. This substance is present in large amounts in extracts of envenomed organs; it is soluble in absolute methyl alcohol and heat stable. Pharmacological actions of alcoholic extract of envenomed monkey's liver ("envenomed liver") have been compared with those of cobra venom and of extract of lecithin treated with venom ("lysocithin").

4. "Envenomed liver" and "lysocithin" cause slow delayed contraction of the guinea-pig's jejunum and characteristic after-changes in reactivity to histamine and to acetylcholine. The effects of cobra venom are similar, but in this case the muscle is readily desensitized.

5. "Envenomed liver", "lysocithin" and cobra venom contract the rat's jejunum, the normal guinea-pig's uterus and the uterus poisoned by histamine. In the case of cobra venom the preparations are readily desensitized.

6. On the isolated cat's heart "lysocithin" causes changes in coronary circulation and strong reduction in the force of the beat; rapid failure occurs and the heart ceases to beat in diastole or in mid-position. Extracts

of normal monkey's liver, which by themselves have no action, protect from the action of "lysocithin", and if the protection is not complete a gradual failure occurs similar to that caused by "envenomed liver". Cobra venom (2–4 mg.) causes rapid failure and systolic contracture of the heart.

7. Injected into the anterior chamber of the rabbit's eye, "envenomed liver", "lysocithin" and cobra venom cause opacity of the cornea and irregular alterations of its curvature. Extracts of normal monkey's liver are without effect.

8. Injected intravenously into guinea-pigs, "envenomed liver" and "lysocithin" cause symptoms resembling acute anaphylactic shock with the addition of haemorrhagic oedema of the lungs.

9. Washed sheep's red corpuscles are immediately haemolysed by envenomed liver and "lysocithin" but not by cobra venom nor by extract of normal monkey's liver. The latter has a protective action against haemolysis by "lysocithin".

10. "Envenomed liver" and "lysocithin" injected into the perfused dog's liver cause output of protein, histamine and pigments. With repeated injections the output of histamine increases. These effects are closely similar to those produced by repeated injections of small doses of cobra venom.

Our thanks are due to Miss F. E. Williams, who carried out numerous haemolysis tests for us.

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